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ARTICLES

Calcium-Modulated Chloride Pathways Contribute to Chloride Flux in Murine Cystic Fibrosis-Affected Macrophages

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ABSTRACT: Cystic fibrosis (CF), a common lethal inherited disorder defined by ion transport abnormalities, chronic infection, and robust inflammation, is the result of mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein, a cAMP-activated chloride (Cl^-) channel. Macrophages are reported to have impaired activity in CF. Previous studies suggest that Cl^- transport is important for macrophage function; therefore, impaired Cl^- secretion may underlie CF macrophage dysfunction. To determine whether alterations in Cl^- transport exist in CF macrophages, Cl^- efflux was measured using *N*-[ethoxycarbonylmethyl]-6-methoxy-quinolinium bromide (MQAE), a fluorescent indicator dye. The contribution of CFTR was assessed by calculating Cl^- flux in the presence and absence of $cftr_{inh}$-172. The contribution of calcium (Ca^{2+})-modulated Cl^- pathways was assessed by examining Cl^- flux with varied extracellular Ca^{2+} concentrations or after treatment with carbachol or thapsigargin, agents that increase intracellular Ca^{2+} levels. Our data demonstrate that CFTR contributed to Cl^- efflux only in WT macrophages, while Ca^{2+}-mediated pathways contributed to Cl^- transport in CF and WT macrophages. Furthermore, CF macrophages demonstrated augmented Cl^- efflux with increases in extracellular Ca^{2+}. Taken together, this suggests that Ca^{2+}-mediated Cl^- pathways are enhanced in CF macrophages compared with WT macrophages. (***Pediatr Res* 70: 447–452, 2011**)

Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene that encodes a protein kinase A (PKA)-activated chloride (Cl^-) channel. In the absence of functional CFTR, defective Cl^- secretion impairs mucociliary clearance and results in viscous secretions in which bacteria proliferate, leading to an influx of immune cells (1,2). The subsequent robust inflammatory response contributes significantly to airway destruction, respiratory failure, and shortened life expectancy. Recent reports suggest that airway inflammation occurs early in life and can be observed before bacterial colonization. Increased numbers of neutrophils and increased IL-8, which may be partially macrophage-derived, have been noted without concomitant infection in the bronchoalveolar lavage (BAL) fluid of infants with CF (3). These findings suggest that if inflammation is present before infection, then macrophages may be important in stimulating the influx of neutrophils into the airways of these patients. Hubeau *et al.* (4) provided additional evidence that macrophages may contribute to this process as they reported increased numbers of macrophages in CF-affected fetal lung tissue in the absence of acute infection or concurrent increase in other immune cells or inflammatory markers. As macrophages are responsible for recruitment of immune cells to sites of inflammation, macrophage dysfunction in CF may result in altered responses to pathogenic stimuli.

If macrophage dysfunction contributes to the robust inflammatory response described in CF, it may be due to impaired Cl^- transport, similar to the mechanism that underlies the pathology observed in CF-affected epithelia. Cl^- flux has been described in macrophages at rest (5,6), during phagocytosis (7), after stimulation when it is associated with increased intracellular calcium (Ca^{2+}) levels (8), and during macrophage activation when it is accompanied by changes in membrane potential. Despite these reports, the exact Cl^- pathways and their roles in macrophage function have not been fully defined.

The aim of this study was to evaluate the Cl^- efflux pathways present in macrophages. More specifically, to define the contributions of CFTR and Ca^{2+}-activated Cl^- pathways to total Cl^- flux. Although CFTR activity has been reported in WT macrophages (9), its functional significance remains a question that requires further investigation. In addition, up-regulation of Ca^{2+}-activated Cl^- channels (CaCCs) has been well described in airway epithelia in the absence of functional CFTR (10), but it is unknown whether this relationship exists in nonepithelial cells. If this relationship is present in macrophages, then it may represent a potential pathway that can be targeted for novel therapeutic intervention. Cl^- efflux was studied in murine bone marrow-derived (BMD) WT and CF macrophages to compare the contribution of these Cl^- efflux pathways to total Cl^- transport.

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Abbreviations: AFU, arbitrary fluorescent units; **BAL,** bronchoalveolar lavage; **BMD,** bone marrow derived; **CaCC,** calcium-activated chloride channel; **CF,** cystic fibrosis; **CFTR,** cystic fibrosis transmembrane conductance regulator; **KSCN,** potassium thiocyanate; **MQAE,** *N*-[ethoxycarbonylmethyl]-6-methoxy-quinolinium bromide; **NPPB,** 5-nitro-2-(3-phenylpropylamino)benzoic acid

METHODS

Animals. For all experiments outlined, two murine models of CF were used: a ΔF508 model ($Cftr^{tm1Kt}$) (11) and a $cftr^{-/-}$ model ($Cftr^{tm1UNC}$) (12). Both models have been fully backcrossed on a Bl6 background. They were bred and maintained as previously described (13–15). All procedures were performed in accordance with protocols approved by the Yale University Institutional Animal Care and Use Committee.

BMD macrophage isolation. BM was obtained from long bones (hip, femur, and tibia) of mice (2–4 mo). Monocytic precursors were selected *via* Histopaque gradient. Following overnight culture, nonadherent macrophages were selectively grown in DMEM media (Invitrogen, Carlsbad, CA) with 10% FCS, L-glutamine, penicillin/streptomycin (100,000 units/mL), and 20 ng/mL recombinant murine macrophage colony stimulating factor (PeproTech Inc., Rocky Hill, NJ). Macrophages were cultured at 37°C with 5% CO_2 for 9–14 d and then harvested with Neutral Protease (Worthington Co., Lakewood, NJ); 5–30 × 10^6 cells were obtained/mouse. Cultured macrophages are F480+/MAC-1+ as confirmed by flow cytometry (16). A suspension of 1 × 10^6 macrophages/mL concentration was used for experiments.

Fluorescent dye indicator studies. Macrophages (~1 × 10^5 cells), attached to glass coverslips precoated with Cell Tak (BD scientific laboratories, San Jose, CA), were incubated for 30 min at 37°C with *N*-[ethoxycarbonylmethyl]-6-methoxy-quinolinium bromide (MQAE, 30 mM) (17). MQAE (Invitrogen, Carlsbad, CA) is a Cl^--sensitive fluorescent indicator dye that measures increases in Cl^- concentration *via* a quenching mechanism. Reductions in cell Cl^- give increases in fluorescent intensity indicative of decreased cytosolic Cl^- concentration (18). Dye loading and subsequent experimentation were performed in a custom perfusion chamber mounted on an Olympus IX-71 inverted microscope (19). MQAE was excited at 354 ± 10 nm, and emitted fluorescent light was measured at 460 ± 10 nm every 5 s using a charge coupled device camera attached to a digital imaging system (20,21). Typically, 10–20 macrophages were monitored simultaneously for each experiment. The rate of change in MQAE fluorescence [Δarbitrary fluorescent units (AFU)/Δtime (s)] was used to calculate Cl^- efflux.

Initially, macrophages were perfused at 3–4 mL/min with Cl^--containing solution [135 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgSO_4$, 2 mM NaH_2PO_4, 2 mM HEPES, and 10 mM glucose or as previously described (20)] to allow for removal of extraneous dye. After the initial perfusion with Cl^--containing buffer, the perfusate was changed to a Cl^--free solution [135 mM NaCyclamate, 3 mM KGluconate, 0.5 mM CaCyclamate, 1.2 mM $MgSO_4$, 2 mM KH_2PO_4, 2 mM HEPES, and 10 mM Glucose or as previously described (20)] in which Cl^- was substituted with cyclamate. In a subset of experiments, the loading of MQAE was assessed by exposing cells to a final perfusion solution containing potassium thiocyanate (KSCN) (150 mM KSCN, 0.5 mM CaCyclamate, 1.2 mM $MgSO_4$, 2 mM KH_2PO_4, 2 mM HEPES, 10 mM Glucose) with Nigericin (10 μM) to measure the minimum specific fluorescence of the cells (20). The control Cl^--free solution contained 0.5 mM Ca^{2+}, which is within the normal range for extracellular Ca^{2+} concentrations (22–25). The high Ca^{2+}/Cl^--free solution had a Ca^{2+} concentration of 2 mM, which can be found in tracheobronchial secretions (26,27). Macrophages were assessed in a low Ca^{2+} (0.1 mM)/Cl^--free solution for comparison. To ensure that the extracellular Ca^{2+} concentrations did not affect cell viability, assays were performed with Trypan blue in each experimental solution demonstrating ≥90% viability. Solutions were adjusted to a final pH of 7.4 at 37°C and an osmolarity of 300 mOsmol.

To confirm the presence of Cl^- movement, macrophages were assessed in the presence of 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB, 100 μM), a broad inhibitor of Cl^- channels (28), as cells transitioned from Cl^--containing to Cl^--free solutions. The contribution of CFTR to total Cl^- flux was evaluated in the presence of the CFTR-specific inhibitor, $cftr_{inh}$-172 (20 μM). Macrophages were treated for 2 min with $cftr_{inh}$-172 in the Cl^--containing solution before assessing Cl^- efflux in the control Cl^--free solution with $cftr_{inh}$-172 still present. Rates of Cl^- efflux after treatment with either inhibitor were compared with rates of Cl^- efflux observed in the absence of the inhibitors. Vehicles alone (ethanol or DMSO) had no effect on efflux.

The effect of increasing intracellular Ca^{2+} concentrations on Cl^- efflux was assessed indirectly after treatment with either carbachol or thapsigargin. Macrophages were treated with carbachol (100 μM) for 30 min, while loading with MQAE (29). Alternatively, macrophages were assessed after treatment with thapsigargin (1 μM) for 2 min in Cl^--containing solution before assessment in Cl^--free solution. After treatment with either agent, Cl^- efflux was assessed in either low Ca^{2+}/Cl^--free solution with addition of EGTA (1 mM) or in the control solution. Chemicals were purchased from Sigma Chemical Co. Corporation unless specified.

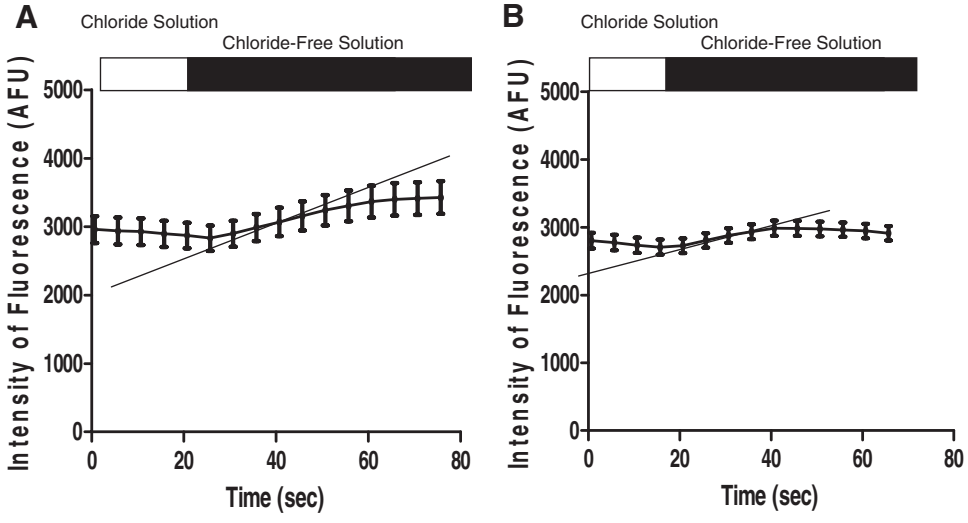

Figure 1. Representative tracings of MQAE fluorescence in WT and $cftr^{-/-}$ macrophages after transition to Cl^--free solution. All macrophages demonstrate an increase in MQAE fluorescence (AFU) in Cl^--free solution as Cl^- exits the cell. The rate of change in fluorescence (*i.e.* slope: ΔAFU/Δs) represents Cl^- efflux (shown with the *gray lines*). The data depict the mean ± SEM values from 20 WT (*A*) and 31 $cftr^{-/-}$ macrophages (*B*).

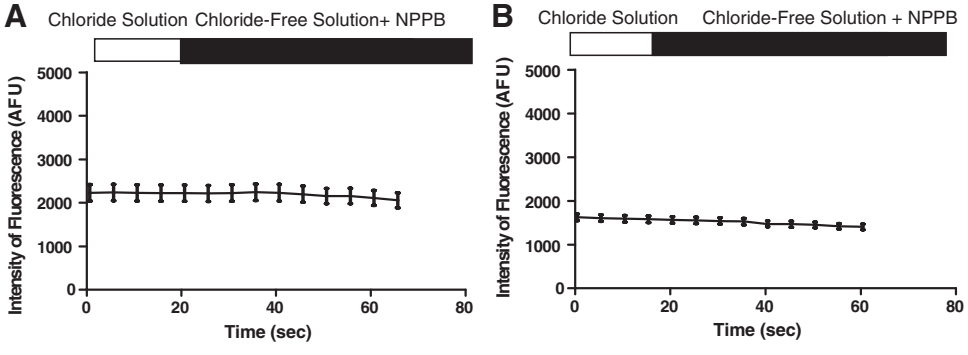

Figure 2. Cl^- efflux is inhibited in WT and CF macrophages by NPPB. These representative tracings from WT (*A*, $n = 5$) and ΔF508/ΔF508 (*B*, $n = 23$) macrophages demonstrate that the addition of NPPB (100 μM) abolishes the expected increase in fluorescence that occurs in Cl^--free solution.

Data analysis. Maximal apparent Cl^- efflux was calculated using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA) in conjunction with Microsoft Excel to compute the first derivative of the change in MQAE fluorescence over time (slope). The data are summarized as the mean Cl^- efflux ($\Delta AFU/\Delta s$) ± the SEM. An unpaired *t* test with Welch's correction (accounting for unequal variances) was performed to compare slopes between experimental conditions. A p value of <0.05 was considered statistically significant.

RESULTS

Chloride efflux is present in both CF-affected and WT macrophages. Changes in MQAE fluorescence in Cl^--containing and control Cl^--free solutions for both WT and CF macrophages are represented in Figure 1. Cl^- efflux, calculated as the rate of change in MQAE fluorescence ($\Delta AFU/\Delta s$), was present in both genotypes. Cl^- efflux was confirmed *via* the absence of flux in both WT and CF macrophages in the presence of NPPB (Fig. 2). The mean Cl^- efflux ($\Delta AFU/\Delta s$) was calculated for comparison between genotypes in control Cl^--free solution (Fig. 3). The Cl^- efflux observed in WT macrophages (9.21 ± 0.51 AFU/s) was significantly greater than CF macrophages (3.22 ± 0.32 AFU/s, $p < 0.0001$). Furthermore, experiments performed with perfusion of KSCN solution demonstrated that MQAE fluorescence in macrophages was 4-fold greater than the background fluorescence.

CFTR contributes to the chloride efflux in macrophages. To assess the specific contribution of functional CFTR to Cl^- efflux, macrophages were studied in the presence and absence of $cftr_{inh}$-172 (20 μM). After exposure to $cftr_{inh}$-172, Cl^- efflux in WT macrophages was significantly reduced (4.6 ± 0.42 AFU/s, $p < 0.0001$) compared with Cl^- efflux observed under control conditions (Fig. 4). In contrast, $cftr_{inh}$-172 had no appreciable effects on Cl^- efflux in CF macrophages (3.9 ± 0.23 AFU/s, $p = 0.09$). Of note, in the presence of $cftr_{inh}$-172, the rate of Cl^- efflux observed in WT macrophages was equivalent to the rate observed in CF macrophages ($p = 0.15$).

The presence of Cl^- efflux in CF and WT macrophages after treatment with $cftr_{inh}$-172 suggests that non–CFTR-dependent Cl^- pathways contributed to total Cl^- efflux in macrophages. Previous studies in CF-affected epithelia have described an up-regulation of CaCCs (10), but this has not been studied in CF macrophages. Therefore, in subsequent experiments, the effects of Ca^{2+} on Cl^- efflux were assessed in macrophages at various extracellular Ca^{2+} concentrations or after treatment with either carbachol or thapsigargin.

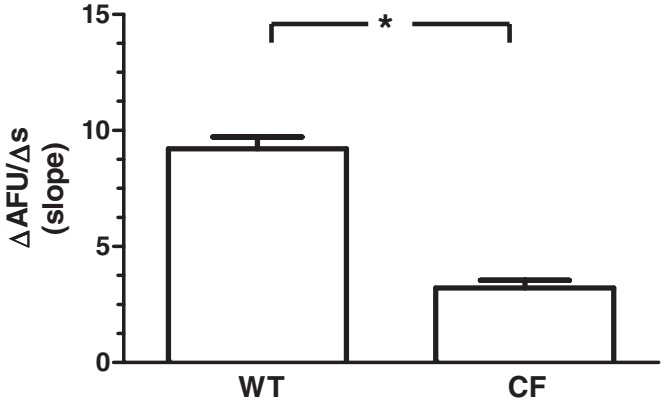

Figure 3. Cl^- efflux under control conditions in WT and CF macrophages. WT macrophages ($n = 157$ from eight mice) demonstrate greater Cl^- efflux compared with CF macrophages ($n = 222$ total from seven ΔF508/ΔF508 mice and seven $cftr^{-/-}$ mice) in control Cl^--free solution. *$p < 0.0001$.

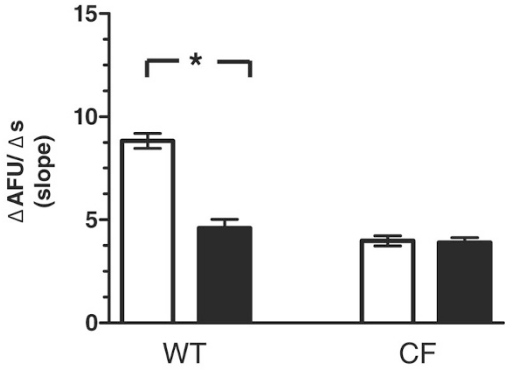

Figure 4. Effects of $cftr_{inh}$-172 on Cl^- efflux in WT and CF macrophages. Cl^- efflux is significantly reduced in WT macrophages ($n = 92$ from six mice) but is unchanged in CF macrophages ($n = 177$ from seven ΔF508/ΔF508 mice and three $cftr^{-/-}$ mice) in the presence of $cftr_{inh}$-172 (■) compared with control conditions (□). * $p < 0.0001$.

Extracellular calcium concentrations increase chloride efflux in CF-affected macrophages. Extracellular Ca^{2+} concentrations affected Cl^- efflux in both WT and CF macrophages. The rates of Cl^- efflux were significantly diminished (1.6 ± 0.22 AFU/s and 2.56 ± 0.23 AFU/s, respectively, $p < 0.0001$) in low Ca^{2+} (0.1 mM) solution compared with rates of Cl^- efflux observed in control (0.5 mM Ca^{2+}) solution as shown in Figure 5*A*. In contrast, only CF-affected macrophages demonstrated a significant increase in Cl^- efflux in high (2 mM) Ca^{2+} solution (6.86 ± 0.4 AFU/s, $p = 0.0002$; Fig. 5*B*). Similar changes were not observed in WT macrophages (7.64 ± 0.67 AFU/s, $p = 0.12$).

Because extracellular Ca^{2+} can ultimately affect intracellular Ca^{2+} levels, the effects of altering intracellular Ca^{2+} concentrations on Cl^- efflux were subsequently assessed. Macrophages were exposed to carbachol, a combined muscarinic and nicotinic receptor agonist that stimulates Ca^{2+} release from intracellular stores (30). In addition, macrophages were exposed to thapsigargin, a sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) pump inhibitor that prevents Ca^{2+} sequestration into the endoplasmic reticulum (31).

Intracellular calcium concentrations modulate chloride efflux in CF macrophages. After treatment with carbachol, only CF macrophages demonstrated a significant augmentation of Cl^- efflux (11.16 ± 0.44 AFU/s, $p < 0.0001$; Fig. 6*A*). The enhanced rate of Cl^- efflux observed in CF macrophages was greater than the rate observed in WT macrophages under control conditions (8.84 ± 0.36 AFU/s, $p < 0.0001$). In contrast, WT macrophages demonstrated a decrease in Cl^- efflux after treatment with carbachol (6.82 ± 0.77 AFU/s, $p = 0.019$).

To confirm that the changes in Cl^- flux observed after carbachol treatment were due to increased intracellular Ca^{2+} concentration rather than nonspecific effects, macrophages were assessed after treatment with thapsigargin. As shown in Figure 6*B*, Cl^- efflux was significantly augmented in CF

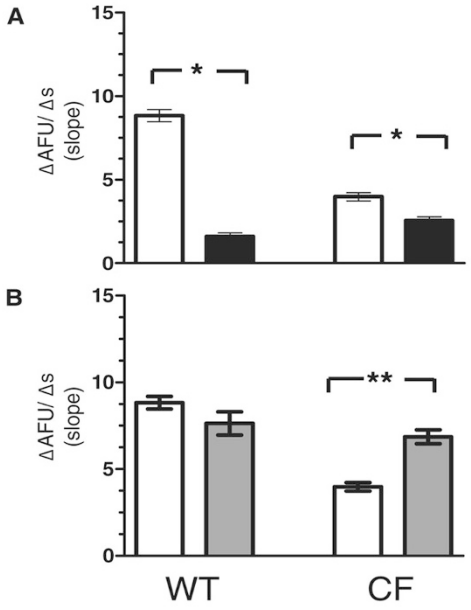

Figure 5. Cl^- efflux in WT and CF macrophages assessed in low and high extracellular Ca^{2+} solutions. (*A*) Both WT ($n = 95$ cells from eight mice) and CF ($n = 213$ cells from seven ΔF508/ΔF508 and seven *cftr*$^{-/-}$ mice) macrophages demonstrate a significant reduction in Cl^- efflux in low Ca^{2+} solution (■) compared with control solution (□). *$p < 0.0001$. (*B*) Cl^- efflux is increased in CF macrophages ($n = 192$ from five ΔF508/ΔF508 and five *cftr*$^{-/-}$ mice) in high Ca^{2+} solution (▩) compared with control solution (□). Similar changes are not present in WT macrophages ($n = 96$ from six mice). **$p = 0.0002$.

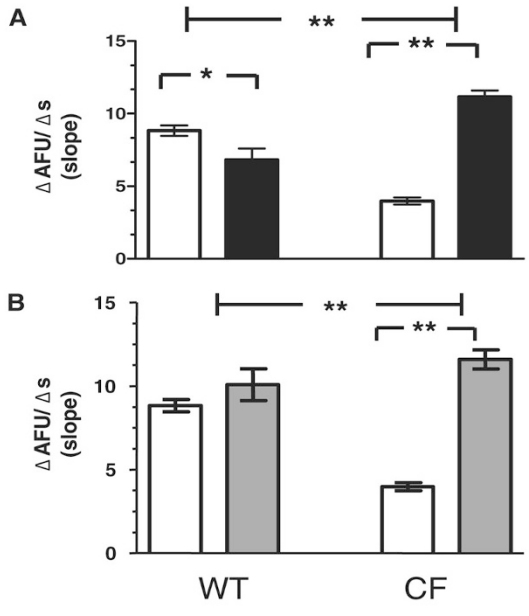

Figure 6. Cl^- efflux following agents that increase intracellular Ca^{2+} concentrations. (*A*) Cl^- efflux in macrophages assessed in control solution after carbachol treatment (■) compared with Cl^- efflux under control conditions alone (□). (WT macrophages = 86 from five mice and CF macrophages = 203 from five ΔF508/ΔF508 and four *cftr*$^{-/-}$ mice.) *$p = 0.019$, **$p < 0.0001$. (*B*) Cl^- efflux in macrophages assessed in control solution after thapsigargin treatment (▩) compared with Cl^- efflux under control conditions alone (□). (WT macrophages = 89 from five mice and CF macrophages = 77 from four ΔF508/ΔF508 and four *cftr*$^{-/-}$ mice.) **$p < 0.0001$. Only CF macrophages demonstrate increases in Cl^- efflux after treatment with either carbachol or thapsigargin.

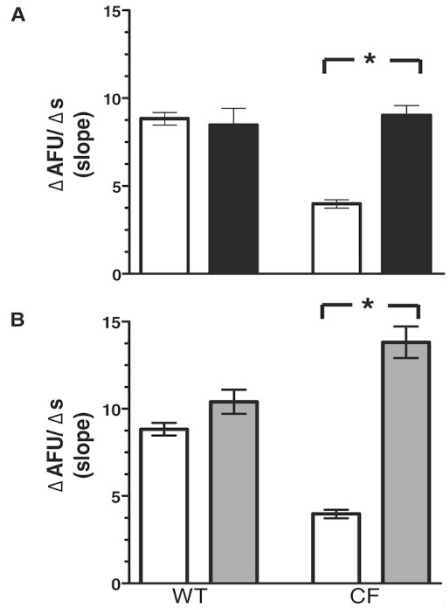

Figure 7. Cl^- efflux assessed in low Ca^{2+} solution. (*A*) Cl^- efflux in macrophages assessed in low Ca^{2+} solution after carbachol treatment (■) compared with Cl^- efflux under control conditions alone (□). (WT = 79 macrophages from four mice and CF = 138 macrophages from four ΔF508/ΔF508 and three *cftr*$^{-/-}$ mice.) *$p < 0.0001$. (*B*) Cl^- efflux in macrophages assessed in low Ca^{2+} solution after thapsigargin treatment (▩) compared with Cl^- efflux under control conditions alone (□). (WT = 27 macrophages from three mice and CF = 130 macrophages from four ΔF508/ ΔF508 and four *cftr*$^{-/-}$ mice.) *$p < 0.0001$. Only CF macrophages demonstrate increases in Cl^- efflux after treatment with either carbachol or thapsigargin.

macrophages after treatment with thapsigargin (11.59 ± 0.57 AFU/s, $p < 0.0001$), providing support that Ca^{2+} mobilized in the setting of SERCA pump inhibition also enhanced Cl^- efflux in these cells. In contrast, WT macrophages demonstrated no change in Cl^- efflux after thapsigargin treatment (10.08 ± 0.95 AFU/s, $p = 0.22$).

Finally, to assess whether Ca^{2+} entry contributed to the observed increase in Cl^- efflux, macrophages were assessed after treatments with either carbachol (Fig. 7*A*) or thapsigargin (Fig. 7*B*), while being perfused with a low extracellular Ca^{2+} solution containing EGTA (1 mM) to chelate external Ca^{2+} available. In the low Ca^{2+} solution, only CF macrophages demonstrated significant enhancement of Cl^- efflux after treatment with carbachol (9.03 ± 0.55 AFU/s, $p < 0.0001$) and thapsigargin (13.82 ± 0.91 AFU/s, $p < 0.0001$). Interestingly, the rate of Cl^- efflux observed in CF macrophages after thapsigargin treatment was greater than that observed in WT macrophages under all conditions studied. In contrast, rates of Cl^- efflux were not similarly increased in WT macrophages after treatment with either carbachol (8.47 ± 0.96 AFU/s, $p = 0.723$) or thapsigargin (10.41 ± 0.69 AFU/s, $p = 0.056$). Taken together, these data suggest that it is unlikely Ca^{2+} entry significantly contributed or modulated the rates of Cl^- efflux observed after carbachol or thapsigargin treatments.

DISCUSSION

CF has been described as a disease of the epithelia (32). However, the possibility that CFTR dysfunction affects non-

epithelial cells, including primary immune cells, has been raised on numerous occasions (1,2). For instance, the BAL specimens from asymptomatic CF infants demonstrate increased levels of IL-8 that could be macrophage-derived (3). In addition, BAL specimens from older patients with CF demonstrate increased numbers of macrophages in combination with increased levels of chemokines, known to attract peripheral monocytes (33). In addition, after stimulation with lipopolysaccharide, CF mice exhibit increased levels of BAL cytokines, that are largely macrophage-derived, compared with WT littermates (16). Moreover, comparable abnormalities of cytokine secretion are observed in their BMD macrophages (16,34). These data suggest that there is a primary defect in CFTR-deficient monocytes that results in their increased activation. Despite these reports, there is no consensus that CFTR dysfunction directly contributes to these findings and thus the role of CFTR in macrophages remains speculative.

Reports suggest that ion channel conductances likely influence immune cell function (35); therefore, Cl^- permeability may play a role in modulating macrophage activities. Previous studies demonstrated that swell-activated (36), voltage-gated (35), and Ca^{2+}-dependent (8) Cl^- pathways are present in macrophages. In addition, the presence of CFTR has been reported in WT macrophages (9). To date, the Cl^- pathways that are present in CF macrophages, where CFTR is absent, are not well-characterized but may play a role in the CF inflammatory response. To our knowledge, this study is the first to compare the contributions of CFTR- and Ca^{2+}-modulated Cl^- pathways to total Cl^- transport in CF and WT macrophages.

Our results demonstrate that although Cl^- efflux is present in both WT and CF macrophages, the contribution of CFTR and other Cl^- pathways to the total Cl^- efflux is different for each genotype. The contribution of CFTR to Cl^- efflux in WT macrophages is demonstrated clearly by the decreased rate of Cl^- efflux observed in WT macrophages treated with $cftr_{inh}$-172. In addition, non–CFTR-dependent Cl^- efflux pathways are present in both CF and WT cells, as each genotype exhibits significant residual flux despite the absence of functional CFTR. Furthermore, these additional pathways are partially mediated by extracellular Ca^{2+} concentrations because a decrease in Cl^- flux is observed in both genotypes when extracellular Ca^{2+} is reduced. Interestingly, only CF macrophages exhibit an increase in Cl^- efflux when extracellular Ca^{2+} concentrations are increased.

The effects of extracellular Ca^{2+} concentrations on Cl^- efflux were unexpected because the link between extracellular Ca^{2+} concentrations and Cl^- flux is not overtly intuitive. It is possible that the presence of divalent cations may stabilize the open state of CFTR and allow for increased Cl^- movement (37) as described previously. This would suggest that altering Ca^{2+} concentration may not only modulate Ca^{2+}-mediated Cl^- pathways but also potentially affect CFTR function in WT macrophages.

An alternative explanation for the effects of extracellular Ca^{2+} on Cl^- efflux may be the presence of Ca^{2+} sensing receptors (CaSR) which have been described in BMD cells (38). Increases in extracellular Ca^{2+} concentrations would activate CaSRs leading to the release of intracellular Ca^{2+} stores (25), subsequently increasing Cl^- flux *via* CaCCs. If this mechanism is present, then increased extracellular Ca^{2+} concentrations will result in an increase in Cl^- efflux, whereas decreased extracellular Ca^{2+} concentrations should have the opposite effect. However, one must also postulate a difference in some portion of this pathway in CF or WT cells as only CF macrophages exhibited an augmentation of Cl^- efflux when examined in high extracellular Ca^{2+} solution. In addition, after modulation of intracellular Ca^{2+} concentrations indirectly with carbachol or thapsigargin, only CF macrophages demonstrated a significant increase in Cl^- efflux. Together, these findings suggest that Ca^{2+} modulates Cl^- secretory pathways in CF and WT macrophages differently.

Interestingly, similar findings have been described in $cftr^{-/-}$ epithelia (10) suggesting Ca^{2+}-modulated Cl^- pathways may represent an alternative route for augmenting Cl^- efflux in the absence of functional CFTR protein in multiple cell types (39–41). One could speculate that under certain circumstances, such as an acute inflammatory response, extracellular Ca^{2+} levels, which range from 1 to 4 mM in tracheobronchial secretions, could result in a more robust Cl^- efflux in these CF macrophages to enhance their function.

However, the use of Ca^{2+}-modulated Cl^- pathways could also be detrimental in the overall CF inflammatory response. For instance, studies indicate that changes in Ca^{2+} mobilization and homeostasis within CF airway epithelia are linked with its predisposition to a hyperinflammatory phenotype (42–45). In addition, Mueller *et al.* (46) recently described that altered intracellular Ca^{2+} mobilization in $cftr^{-/-}$ lymphocytes led to the induction of inflammatory signaling pathways and cytokine secretion. Thus, the enhancement of Ca^{2+}-modulated Cl^- efflux pathways in our CF macrophages may be a potential mechanism by which macrophages directly contribute to the hyperinflammatory phenotype and airway pathophysiology observed in CF.

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